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APPLICANTS

MICHAEL C. PIRRUNG, MENLO PARK, CA; J. LEIGHTON READ, PALO ALTO, CA.

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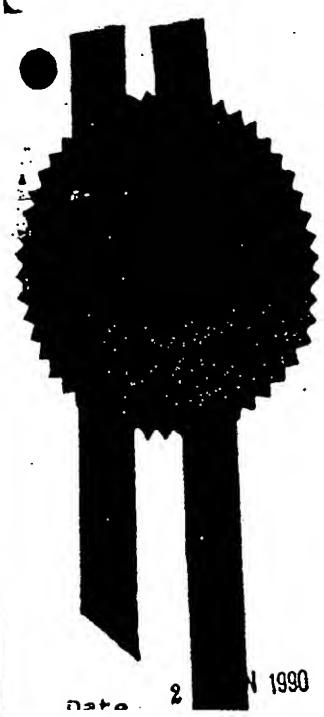
ADDRESS

VERN NORVIEL
TOWNSEND & TOWNSEND
STEUART STREET TOWER, ONE MARKET PLAZA
SAN FRANCISCO, CA 94105

TITLE

VERY LARGE SCALE IMMOBILIZED POLYMER SYNTHESIS



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PATENT APPLICATION SERIAL NO. 07/362901

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JUN 7 1989
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Be it known that we, MICHAEL C. PIRRUNG, a resident of the City of Menlo Park, County of San Mateo, State of California, and J. LEIGHTON READ, a resident of the City of Palo Alto, County of Santa Clara, State of California, both citizens of the United States of America, have invented new and useful improvements in

VERY LARGE SCALE IMMOBILIZED POLYMER SYNTHESIS

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VERY LARGE SCALE IMMOBILIZED POLYMER SYNTHESIS

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to the field of polymer synthesis. In particular, the present invention provides a method and associated apparatus for preparing a large variety of known chemical sequences at known locations on a single substrate surface. The method may be applied, for example, in the field of preparation of oligomers, peptides, nucleic acids, oligosaccharides, phospholipids, polymers, and mixtures thereof, especially to create sources of chemical diversity for use in screening for biological activity.

Description of Related Art

The relationship between structure and activity of molecules is a fundamental issue in the study of biological systems. Structure-activity relationships (SAR) are important in understanding the function of enzymes, the ways in which cells communicate with each other, as well as cellular control and feedback systems.

Certain macromolecules are known to interact and bind to other molecules having a very specific three-dimensional distribution of charge in space. Any large molecule having such specificity can be considered a receptor, whether it is an enzyme catalyzing hydrolysis of a metabolic intermediate, a cell-surface protein mediating membrane transport of ions, a glycoprotein serving to identify a particular cell to its neighbors, an IgG-class antibody circulating in the plasma, or an oligonucleotide sequence of DNA in the nucleus. The various molecules to which receptors selectively bind are known as ligands.

Many assays are available for measuring the binding affinity of known receptors and ligands, but the information which can be gained from such experiments is

screening. For example, the "Merrifield" method (J. Am. Chem. Soc. (1963) 85:2149-2154), which is incorporated herein by reference for all purposes, has been used to synthesize peptides on a solid support. In the Merrifield method, an amino acid is covalently bonded to a support made of an insoluble polymer. Another amino acid with an alpha protected group is reacted with the covalently bonded amino acid to form a dipeptide. After washing, the protective group is removed and a third amino acid with an alpha protective group is added to the dipeptide. This process is continued until a peptide of a desired length is obtained. Using the Merrifield method, it is not economically practical to synthesize more than a handful of peptide sequences in a day.

To synthesize larger numbers of polymer sequences, it has also been proposed to use a series of reaction vessels for polymer synthesis. For example, a tubular reactor system may be used to synthesize a linear polymer on a solid phase support by automated sequential addition of reagents. This method enables the practical synthesis of a larger number of sequences than the method provided by Merrifield, but still does not enable the synthesis of a sufficiently large number of polymer sequences for effective economical screening.

Methods of preparing a plurality of polymer sequences are also known in which a foraminous container encloses a known quantity of reactive particles, the particles being larger in size than foramina of the container. The containers may be selectively reacted with desired ligands to synthesize desired sequences of product molecules. As with other methods known in the art, this method cannot practically be used to synthesize a sufficient variety of polypeptides for effective screening.

Techniques which have been previously described allowed a change in the scale in which standard Merrifield solid-phase synthesis could take place from dozens

of peptides a week to hundreds or thousands. These include the simultaneous synthesis of peptides on 96 plastic pins which fit the format of standard microtiter plates.

In connection with this change in the scale of synthetic diversity, a general technique has been proposed for finding a catamer which binds with high affinity to another molecule which relies on an iterative sequence of synthesis and analysis, building up from smaller to larger catamers. This so-called "mimotope strategy" has been best described in the context of peptide synthesis. This approach begins with the synthesis of all 400 of the possible dipeptides (in some cases at the amino terminal end of the linker attached to a pin; in other cases, imbedded in a larger peptide with the adjacent amino acids not specified, but varying randomly). Resulting peptides are then contacted with a monoclonal antibody or other receptor in an ELISA or other assay, and those found to have relatively higher binding affinities are selected for extension in the next step. The high affinity dipeptides are resynthesized along with adjacent amino acids which are systematically varied to create all of the possible tripeptides containing the previously selected dipeptide sequences. These are then assayed for binding and those having the highest affinity are chosen for the tetrapeptide extension. These steps are repeated until a peptide of 6-8 amino acids having the desired affinity is found.

Many variations and refinements have been proposed in the hopes of allowing discovery of ligands having particular characteristics, including syntheses using unnatural amino acids and inclusion of monomers with specific stearic properties. Unfortunately, while these techniques have been somewhat useful, substantial problems remain. One important problem in the mimotope strategy is that binding affinities at the dipeptide and tripeptide levels may lead to "blind alleys" in which the

subsequent extension steps lead to a reduction rather than an increase in binding affinity. Moreover, pursuing this strategy can be time consuming and uneconomical.

From the above, it can be seen that an improved method and apparatus for synthesizing a variety of chemical sequences at known locations is desired.

SUMMARY OF THE INVENTION

An improved method and apparatus for the preparation of polymers is disclosed. The method and apparatus may be applied to synthesize a variety of polymers at known locations on a substrate. The method could be used to synthesize up to about 10^6 or more different sequences per cm^2 at known locations in some embodiments.

In one embodiment, linker molecules are provided on a substrate. A terminal end of the linker molecules is provided with a reactive functional group protected with a photoremovable protective group. Using optical methods, the photoremovable protective group is exposed to light and removed from the linker molecules at first selected locations. The substrate is then washed or otherwise contacted with a first monomer that reacts with exposed functional groups on the linker molecules. In a preferred embodiment, the chemical subunit is an amino acid containing a photoremovable protective group at its amino terminus and the linker molecule terminates in an amino acid group bearing a photoremovable protective group.

A second set of selected locations is, thereafter, exposed to light and the photoremovable protective group on the linker molecule/protected amino acid is removed at the second set of locations. The substrate is then contacted with a second amino acid containing a photoremovable protective group. This process is repeated to expose and selectively apply monomers until polymers of a desired length and desired chemical se-

quence are obtained. Protective groups on the resulting sequences are, thereafter, removed and the sequence is capped. In alternative embodiments an electrochemically removable protective group is used. The protective groups are then removed by selective activation of electrodes underlying the protective groups.

By using lithographic techniques readily known, for example, in the semiconductor industry, it is possible to direct light to relatively small and precisely known locations on the substrate. It is, therefore, possible to synthesize oligomers of a known chemical sequence at known locations on the substrate.

The resulting substrate will have a variety of uses including, for example, screening large numbers of polymers for biological activity. The substrate is exposed to one or more receptors, each labeled with, for example, a radioactive marker, or a labeled antibody reactive with the receptor. The location of the marker on the substrate is detected with, for example, autoradiographic techniques. By knowing the sequence of the material at the location where binding is detected, it is possible to quickly determine which sequence is reactive with the receptor and, therefore, screen large numbers of peptides.

In some applications, the method and apparatus result in order-of-magnitude productivity improvements in the synthesis of oligomers.

Accordingly, in one embodiment, the invention provides apparatus for preparation of a plurality of sequences comprising a substrate with a surface, the surface comprising a reactive portion, the reactive portion activated upon exposure to an energy source so as to react with a monomer of the sequence; and means for selectively protecting and exposing portions of the surface from the energy source.

In another embodiment, the invention provides a method of preparing a substrate comprising the steps of exposing a first area of the substrate to an activator to remove a protective group; exposing at least the first area to a first monomer; exposing a second area to an activator to remove a protective group; and exposing at least the second area to a second monomer.

The method enables greater ease in peptide synthesis because the physical separation of reagents is not required when growing polymer chains. The chains themselves are separated by different physical locations on the substrate, but the entire substrate is exposed to the various reagents as the synthesis is conducted. Differential reaction is achieved by selectively exposing reactive functional groups to, e.g., light, electric currents, or another spatially localized activator. Remaining areas on the substrate remain unreacted.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 illustrates masking and irradiation of a substrate at a first location. The substrate is shown in cross-section.

Figure 2 illustrates the substrate after application of a subunit "A."

Figure 3 illustrates irradiation of the substrate at a second location.

Figure 4 illustrates the substrate after application of subunit "B."

Figure 5 illustrates irradiation of the "A" subunit.

Figure 6 illustrates the substrate after a second application of "B."

Figure 7 illustrates the completed substrate.

Figures 8a-8m illustrate the method as it is applied to the production of the trimers of monomers "A" and "B."

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

CONTENTS

I. Glossary

The following terms are intended to have the following general meanings as they are used herein:

1. Catamer: A polymer molecule which is a defined linear sequence formed by the joining of small molecules. This term includes molecules in which different types of chemical reactions, such as covalent bond formation (e.g., peptide bonds), are used to join the small molecules.

2. Complementary: Refers to the matching together of the reacting surfaces of a ligand molecule and its receptor. Thus, the receptor and its ligand can be described as complementary, and furthermore, the contact surface characteristics are complementary to each other.

3. Epitope: The portion of an antigen molecule which is delineated by the area of interaction with the subclass of receptors known as antibodies.

4. Ligand: The molecule which is recognized, or is bound, by a particular receptor and when bound may mediate or antagonize the biological function associated with that particular receptor.

Examples of ligands which can be investigated by this invention include, but are not restricted to:

a) Agonists or antagonists for cell membrane receptors: For example, neurotransmitters, peptide hormones, growth factors and the like which may react with specific cell surface receptors which mediate a wide range of cellular events via various chemical signal mechanisms. Also, the method may be used to determine which catamers bind with high affinity to these receptors and this may lead to the development of drugs which activate these receptors or antagonize endogenous ligands to produce desired pharmacological effects.

b) Toxins and venoms: For instance, the combining site of the toxin molecule which reacts with a particular receptor in the body to give the particular symptom(s) of intoxication; determination of catamers which bind with high affinity to the combining site of the ligand may lead to the development of drugs which can be used to treat envenomation by snakes and other poisonous animals without the side effects of heterologous antivenins.

c) Virus and other microorganism capsid molecules: For instance, the combining site on the virus coat molecule which reacts with a particular receptor on the cell membrane in the body and which allows the virus to invade and thus infect the particular cell; determination of catamers which bind with high affinity to this combining site may lead to the development of

drugs which specifically prevent intracellular invasion by the virus and thus prevent their replication.

5. Monomer: A member of the set of small molecules which can be joined together to form a polymer. The set of monomers includes but is not restricted to, for example, the set of common L-amino acids, the set of D-amino acids, the set of synthetic amino acids, the set of nucleotides and the set of pentoses and hexoses.

6. Peptide: A polymer in which the monomers are alpha-amino acids and which are joined together through a peptide bond. In the context of this specification it should be appreciated that the amino acids may be the L-optical isomer or the D-optical isomer. Often, peptides are less than 20 amino acids long.

7. Radiation: Energy having a wavelength of between 10^{-14} and 10^{4} meters including, for example, electron beam radiation, gamma radiation, x-ray radiation, ultra-violet radiation, visible light, infrared radiation, microwave radiation, and radio waves. "Irradiation" refers to the application of radiation to a surface.

8. Receptor: A molecule or molecular complex which will combine specifically with its particular ligand molecule. It is those receptors which on binding with their particular ligand(s) mediate a biological function that are of most interest. Examples of receptors include, but are not restricted to, the common class of receptors associated with the surface membrane of cells and include, for instance, the immunologically important receptors of B-cells, T-cells, macrophages and the like. Another example

is receptors for acetylcholine on nerve cells which cause a nerve impulse to be transmitted across a synapse down the length of the neuron when the receptor molecule reacts with its ligand, acetylcholine.

Examples of receptors which can be investigated by this invention include but are not restricted to:

a) Hormone receptors: For instance, the receptors for insulin and growth hormone; determination of the ligands which bind with high affinity to a receptor is useful in the development of an oral replacement of the daily injections which diabetics must take to relieve the symptoms of diabetes, and in the other case, a replacement for the scarce human growth hormone which can only be obtained from cadavers or by recombinant DNA technology. Other examples are the vasoconstrictive hormone receptors; determination of those ligands which bind to a receptor may lead to the development of drugs to control blood pressure.

b) Opiate receptors: Determination of receptors which bind to ligands binding to the opiate receptors in the brain is useful in the development of less-addictive replacements for morphine and related drugs.

c) Microorganism receptors: Determination of ligands which bind to receptors, such as specific transport proteins or enzymes essential to survival of microorganisms, is useful in a new class of antibiotics. Of particular value would be antibiotics against opportunistic fungi, protozoa, and those bacteria resistant to the antibiotics in current use.

d) Enzymes: For instance, the binding site of enzymes such as the enzymes responsible for cleaving neural transmitters; determination of

ligands which bind to certain receptors to modulate the action of the enzymes which cleave the different neural transmitters is useful in the development of drugs which can be used in the treatment of disorders of neural transmission.

e) Antibodies: For instance, the invention may be useful in investigating the ligand-binding site on the antibody molecule which combines with the epitope of an antigen of interest; determining a sequence that mimics an antigenic epitope may lead to the development of vaccines of which the immunogen is based on one or more of such sequences or lead to the development of related diagnostic agents or compounds useful in therapeutic treatments such as for autoimmune diseases (e.g., by blocking the binding of the "self" antibodies).

II. General

The present invention provides methods and apparatus for the preparation and use of a substrate having a plurality of different polymers at predetermined locations. The invention is described herein primarily with regard to the preparation of molecules containing a sequence of monomer subunits, such as amino acids, but could readily be applied in the preparation of other polymers. Such linear and cyclic polymers might include nucleic acids, oligosaccharides, phospholipids, and peptides having either α-, β-, or ω-amino acids, heteropolymers in which a known drug is covalently bound to any of the above, polyurethanes, polyesters, polycarbonates, polyureas, polyamides, polyethyleneimines, polyarylene sulfides, polysiloxanes, polyimides, polyacetates, or other polymers which will be apparent upon review of this disclosure. In a preferred embodiment, the invention herein is used in the synthesis of polypeptides.

The prepared substrate may, for example, be used in screening a variety of ligands for binding with a receptor. The substrate disclosed herein will have a wide variety of other uses. Merely by way of example, the invention herein can be used in determining peptide and nucleic acid sequences which bind to proteins, identifying epitopes recognized by antibodies, and evaluation of a variety of drugs for clinical and diagnostic applications, as well as combinations of the above.

The invention preferably provides for a substrate "S" with a surface. Linker molecules "L" are optionally provided on a surface of the substrate. The purpose of the linker molecules, in some embodiments, is to provide greater exposure of the synthesized polymers to materials to which the polymers are later exposed in screening. On the substrate or a terminal end of the linker molecules a functional group with a protective group P_1 is provided. The protective group P_1 may be removed upon exposure to radiation, electric fields, electric currents, or other activators to expose the functional group.

In a preferred embodiment, the radiation is UV, IR, or visible light. As more fully described below, the protective group may alternatively be an electrochemically-sensitive group which may be removed in the presence of an electric field. In still further alternative embodiments, ion beams, electron beams, or the like may be used as an activator to deprotect the end monomer.

In some embodiments, the exposed area is less than about 1 cm^2 or less than 1 mm^2. In preferred embodiments the exposed area is less than about 10,000 μm^2 or, more preferably, less than 100 μm^2.

Concurrently or after exposure of a known portion of the substrate to light, the surface is contacted with a first monomer unit M_1 which reacts with the functional group. The first monomer includes a protective group P_2. P_2 may or may not be the same as P_1.

Accordingly, after a first cycle, known first portions of the surface may comprise the sequence:

$$S\text{-}L\text{-}M_1\text{-}P_2$$

while remaining portions of the surface comprise the sequence:

$$S\text{-}L\text{-}P_1.$$

Thereafter, second portions of the surface (which may include the first portion) are exposed to light and contacted with a second monomer M_2 (which may or may not be the same as M_1) having a protective group P_3. P_3 may or may not be the same as P_1 and P_2. After this second cycle the substrate may comprise one or more of the following sequences:

$$S\text{-}L\text{-}M_1\text{-}M_2\text{-}P_3$$
$$S\text{-}L\text{-}M_2\text{-}P_3$$
$$S\text{-}L\text{-}M_1\text{-}P_2 \text{ and/or}$$
$$S\text{-}L\text{-}P_1.$$

The above process is repeated until the substrate includes desired polymers of desired lengths. By controlling the locations of the substrate exposed to light, the location of each sequence may be known.

Thereafter, the protective groups are removed from some or all of the substrate and the sequences are, optionally, capped with a capping unit C. The process results in a substrate having a surface with a plurality of polymers of the following general formula:

$$S\text{-}[L]\text{-}(M_i)\text{-}(M_j)\text{-}(M_k)\ldots(M_x)\text{-}[C]$$

where square brackets indicate optional groups, and $M_i \ldots M_x$ indicate any set of monomers. The number of

monomers could cover a wide variety of values, but in a preferred embodiment they will range from 2 to 100 with a most preferred range of about 3 to 8.

In some embodiments a plurality of locations on the substrate are to contain sequences having a common subsequence. For example, it may be desired to synthesize a sequence $S-M_1-M_2-M_3$ at first locations and a sequence $S-M_4-M_2-M_3$ at second locations. The process would commence with irradiation of the first locations followed by contacting with M_1-P, resulting in the sequence $S-M_1-P$ at the first location. The second locations would then be irradiated and contacted with M_4-P, resulting in the sequence $S-M_4-P$ at the second locations. Thereafter both the first and second locations could be irradiated and contacted with the dimer M_2-M_3, resulting in the sequence $S-M_1-M_2-M_3$ at the first locations and $S-M_4-M_2-M_3$ at the second locations. Of course, common subsequences of any length could be utilized including those in a preferred range of 2 to 100 monomers and a most preferred range of 2-3 monomers.

The polymers prepared on a substrate according to the above methods will have a variety of uses including, for example, screening for biological activity. In such screening activities, the substrate containing the sequences is exposed to a labeled receptor. The receptor may bind with one or more polymers on the substrate. The presence of the labeled receptor and, therefore, the presence of a sequence which binds with the antibody is detected in a preferred embodiment through the use of autoradiography, detection of fluorescence with a charge-coupled device, fluorescence microscopy, or the like. The sequence at the locations where the receptor is detected may be used to determine all or part of a sequence which is complementary to the receptor.

III. Substrate Preparation

Figure 1 illustrates one embodiment of the invention disclosed herein in which a substrate 2 is shown in cross-section. The substrate may be in the shape of a disc, square or any other convenient shape. The substrate and its surface 4 preferably form a rigid support on which to carry out the reactions described herein. The substrate and its surface should also be chosen to provide appropriate light-absorbing characteristics. As examples, the substrate may be a polymerized Langmuir Blodgett film, functionalized glass, Si, Ge, GaAs, GaP, SiO_2, SiN_4, modified silicon, or any one of a wide variety of polymers such as (poly)tetrafluoroethylene, (poly)vinylidenedifluoride, or combinations thereof. Other substrate materials will be readily apparent to those of skill in the art upon review of this disclosure. In a preferred embodiment the substrate is flat glass or single-crystal silicon with surface features of less than 10 Å.

The surface 4 of the substrate is preferably provided with a layer of linker molecules 6, although it will be understood that the linker molecules are not required elements of the invention. The linker molecules are preferably of sufficient length to permit polymers in a completed substrate to interact freely with molecules exposed to the substrate. The molecules should be 6-50 atoms long to provide sufficient exposure. The linker molecules may be, for example, aryl acetylene, ethylene glycol oligomers containing 2-10 monomer units, diamines, diacids, amino acids, or combinations thereof. Other linker molecules may be used in light of this disclsoure.

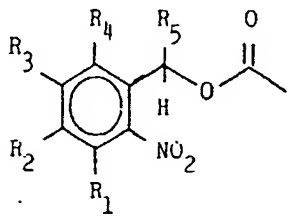
The linker molecules can be attached to the substrate via carbon-carbon bonds using, for example, (poly)trifluorochloroethylene surfaces, or preferably, by siloxane bonds (using, for example, glass or silicon oxide surfaces). Siloxane bonds may be formed via reactions of linker molecules bearing trichlorosilyl

groups with the surface of the substrate. The linker molecules may optionally be attached in an ordered array, i.e., as parts of the head groups in a polymerized Langmuir Blodgett film.

A. Protective Groups

The linker molecules are provided with a functional group protected by a reactive group. Preferably, the reactive group is on the terminal end of the linker molecule opposite the substrate. The reactive group may be either a negative reactive group (i.e., the reactive group renders the linker molecules less reactive with a monomer upon exposure) or a positive reactive group (i.e., the reactive group renders the linker molecules more reactive with a monomer upon exposure). In the case of negative reactive groups an additional step of reactivation will be required. In some embodiments, this will be done by heating.

The protective/reactive group on the linker molecules may be selected from a wide variety of positive light-reactive groups preferably including nitro aromatic compounds such as o-nitrobenzyl derivatives or benzylsulfonyl. In a preferred embodiment, 6-nitroveratryloxycarbonyl (NVOC), 2-nitrobenzyloxycarbonyl (NBOC) or α,α-dimethyl-dimethoxybenzyloxycarbonyl (DDZ) may be used in some embodiments. In general, a nitro aromatic compound containing a benzylic hydrogen ortho to the nitro group may be used, i.e., chemical of the form:



... [monomer or link]

where R_1 is alkoxy, alkyl, halo, aryl, alkenyl, or hydrogen; R_2 is alkoxy, alkyl, halo, aryl, nitro, or hydrogen; R_3 is alkoxy, alkyl, halo, nitro, aryl, or hydrogen; R_4 is alkoxy, alkyl, hydrogen, aryl, halo, or nitro; and R_5 is alkyl, alkinyl, cyano, alkoxy, hydrogen, halo, aryl, or alkenyl. Other materials which may be used include o-hydroxy-α-methyl cinnamoyl derivatives. Photoremovable protective groups are described in, for example, Patchornik, J. Am. Chem. Soc. (1970) 92:6333 and Amit et al., J. Org. Chem. (1974) 39:192, both of which are incorporated herein by reference.

In an alternative embodiment the positive reactive group is activated for reaction with reagents in solution. For example, a 5-bromo-7-nitro indoline group, when bound to a carbonyl, undergoes reaction upon exposure to light at 420 nm.

In a second alternative embodiment, the reactive group on the linker molecule may be selected from a wide variety of negative light-reactive groups including a cinammate group.

Alternatively, the reactive group may be activated or deactivated by electron beam lithography, x-ray lithography, or any other radiation. Suitable reactive groups for electron beam lithography include sulfonyl. Other methods may be used including, for example, exposure to a current source. Other reactive groups and methods of activation may be used in light of this disclosure.

B. Light Exposure

As shown in Figure 1, the linking molecules are preferably exposed to, for example, light through a suitable mask 8 using photolithographic techniques of the type readily known in the semiconductor industry and described in, for example, Sze, "VLSI Technology," McGraw-Hill (1983), which is incorporated herein by reference. Figure 1 illustrates the use of such masking

techniques as they are applied to a positive reactive group so as to activate linking molecules and expose functional groups in areas 10a and 10b.

The mask 8 is in one embodiment a transparent support material coated with a layer of opaque material. Portions of the opaque material are removed, leaving opaque material in the precise pattern desired on the substrate surface. The mask is brought into close proximity with or directly into contact with the wafer surface as shown in Figure 1. "Openings" in the mask correspond to locations on the substrate where it is desired to remove photoremovable protective groups from the substrate. Alignment may be performed using conventional alignment techniques in which alignment marks (not shown) are used to accurately overlay successive masks with previous patterning steps, or more sophisticated techniques may be used. For example, interferometric techniques such as the one described in Flanders et al., "A New Interferometric Alignment Technique," App. Phys. Lett. (1977) 31:426-428, which is incorporated herein by reference, may be used.

To enhance constrast of light applied to the substrate it may be desirable to provide contrast enhancement materials between the mask and the substrate. This contrast enhancement layer may comprise a molecule which is decomposed by light such as quinone diazid.

The light may be from a conventional incandescent source, a laser, or the like. If non-coherent sources of light are used it may be desirable to provide a thick- or multi-layered mask to prevent spreading of the light onto the substrate. Lasers may be preferable because they can more easily provide wavelengths particularly suited for a chromophore of the photosensitive group. It may, further, be desirable in some embodiments to utilize groups which are sensitive to different wavelengths to control synthesis. For example, by using groups which are sensitive to different wavelengths,

it would be possible to select branch positions in the synthesis of a polymer. Several reactive groups along with their corresponding wavelengths for deprotection are provided in Table 1.

Table 1

Group	Deprotection Wavelength
Nitroveratryloxy carbonyl	UV (300-350 nm)
Nitrobenzyloxy carbonyl	UV (300-350 nm)
Dimethyl dimethoxybenzyloxy carbonyl	UV (280-300 nm)
5-Bromo-7-nitroindolinyl	UV (420 nm)
o-Hydroxy-α-methyl cinnamoyl	UV (300-350 nm)
2-Oxymethylene anthraquinone	UV (350 nm)

While the invention is illustrated primarily herein by way of the use of a mask to illuminate the substrate, other techniques may also be used. For example, the substrate may be rotated under modulated laser or diode light source. Such techniques are discussed in, for example, U.S. Patent No. 4,719,615 (Feyrer et al.), which is incorporated herein by reference.

The substrate may be irradiated either in contact or not in contact with a solution (not shown) and is, preferably, irradiated in contact with a solution. The solution may contain reagents to prevent the by-products formed from irradiation from interfering with synthesis of the oligomer. Such by-products might include, for example, carbon dioxide, nitrosocarbonyl compounds, styrene derivatives, indole derivatives, and products of their photochemical reactions. Reagents added to the solution may include, for example, acidic or basic buffers, thiols, substituted hydrazines and hydroxylamines, reducing agents (e.g., NADH) or reagents known to react with a given functional group (e.g., aryl nitroso + glyoxylic acid → aryl formhydroxamate + CO_2).

Either concurrently with or after the irradiation step, the linker molecules are washed or otherwise contacted with a first monomer, illustrated by "A" regions 12a and 12b in Figure 2. The first monomer reacts with the activated functional groups of the linkage molecules which have been exposed to light. The first monomer, which is preferably an amino acid is also provided with a photoprotective group. The photoprotective group on the monomer may be the same as or different than the protective group used in the linkage molecules, and may be selected from any of the above-described protective groups. In one embodiment, the protective groups for the A monomer may be selected from the group NBOC or NVOC.

As shown in Figure 3, the process of irradiating is thereafter repeated, with a mask positioned so as to remove linkage protective groups and expose functional groups in regions 14a and 14b which are illustrated as being regions which are protected in the previous masking step. In alternative embodiments, some steps may provide for successively illuminating a common region in successive steps. As shown in Figure 3, it may be desirable to provide separation between irradiated regions. For example, separation of about 1-5 μm may be appropriate to account for alignment tolerances.

As shown in Figure 4, the substrate is then exposed to a second protected amino acid "B," producing B regions 16a and 16b. Thereafter, the substrate is again masked so as to remove the protective groups and expose reactive groups on A region 12a and B region 16b. The substrate is again exposed to amino acid B, with the resulting structure shown in Figure 6. As shown, the dimers B-A and B-B have been produced on the substrate.

A subsequent series of masking and contacting steps similar to those described above with A (not shown) provides the structure shown in Figure 7. The process

provides all possible dimers of B and A, i.e., B-A, A-B, A-A, and B-B.

The density of polymers on the substrate will be limited by the density with which the surface can be functionalized. This usually does not exceed one functional group per 15x15 Å square. This corresponds to $>4x10^5$ molecules/1 μm^2. By comparison, an IgG antibody has an average footprint of ca. 15 nm^2. Therefore, $4x10^3$ antibodies may be packed into a 1 μm^2 area. The oligomer will therefore be provided in a large (100x) excess. Assuming 10x10 μm squares of oligomers are utilized, up to $4x10^5$ antibody molecules may be bound per square. Autoradiography may be used to detect as few as $4x10^5$ antibody molecules. Fluorescence may be used in some embodiments to detest as few as 1000 molecules. Therefore, the method described herein could be used to study up to 1 million different oligomers, each in a 10 μm square, on a substrate size of 1 cm square. The area of synthesis is preferably less than 1 cm^2. The substrate, the area of synthesis, and the area for synthesis of each individual polymer could be of any size or shape. For example, squares, rectangles, or circles may be utilized. Multiple synthesis areas may also be applied to a substrate.

In one embodiment the regions 12 and 16 on the substrate will have a surface area of between about 1 cm^2 and $1x10^{-8}$ cm^2. In a preferred embodiment, the regions 12 and 16 will have an area of between about 10x10 μm and 100x100 μm.

The irradiated area may take on any one of a variety of shapes. For example, the irradiated area may be circles, squares, ellipsoids, or any irregular geometric shape.

IV. Trimer and Dimer Synthesis

Figure 8 illustrates a possible synthesis of the eight trimers of the two-monomer set: gly, phe (represented by "A" and "B," respectively). A glass slide bearing silane groups terminating in 6-nitroveratryloxycarboxamide (NVOC-NH) residues is prepared as a substrate. Active esters (pentafluorophenyl, OBt, etc.) of gly and phe protected at the amino group with NVOC are prepared as reagents. While not pertinent to this example, if side chain protecting groups are required for the monomer set, these must not be photoreactive at the wavelength of light used to protect the primary chain.

For a monomer set of size n, n x ℓ cycles are required to synthesize all possible sequences of length ℓ. A cycle consists of:

1. Irradiation through an appropriate mask to expose the amino groups at the sites where the next residue is to be added, with appropriate washes to remove the by-products of the deprotection.
2. Addition of a single activated and protected (with the same photochemically-removable group) monomer unit, which will react only at the sites addressed in step 1, with appropriate washes to remove the excess reagent from the surface.

The above cycle is repeated for each member of the monomer set until each location on the surface has been extended by one residue in one embodiment. In other embodiments, several residues are sequentially added at one location before moving on to the next location. Cycle times will generally be limited by the coupling reaction rate, now as short as 20 min in automated peptide synthesizers. For some types of polymers (e.g., peptides), a final deprotection of the entire surface followed by capping (eliminating reactive functional

groups) may be required to stabilize the array for testing.

More particularly, as shown in Figure 8a, the glass 20 is provided with regions 22, 24, 26, 28, 30, 32, 34, and 36. Regions 30, 32, 34, and 36 are masked, as shown in Figure 8b and the glass is irradiated and exposed to "A" (e.g., gly), with the resulting structure shown in Figure 8c. Thereafter, regions 22, 24, 26, and 28 are masked, the glass is irradiated (as shown in Figure 8d) and exposed to "B" (e.g., phe), with the resulting structure shown in Figure 8e. The process proceeds, consecutively masking and exposing the sections as shown until the structure shown in Figure 8m is obtained. The glass is irradiated and the terminal groups are capped by acetylation. As shown, all possible trimers of gly/phe are obtained.

In this example, no side chain protective group removal is necessary. If it is desired, this may be accomplished by treatment with ethanedithiol and trifluoroacetic acid.

In general, the number of steps needed to obtain a particular polymer chain is defined by:

$$n \times \ell \quad (1)$$

where:

n = the number of possible monomers, and

ℓ = the number of monomers in the polymer chain.

Conversely, the number of sequences obtained will be:

$$n^{\ell}. \quad (2)$$

The number of masks needed will generally be n for each "layer" of monomers, i.e., the total number of masks (and, therefore, the number of lithographic steps)

needed will be n x ℓ. The size of the transparent areas will vary in accord with the area available for synthesis and then number of sequences to be used. In general, the size of the synthesis areas will be:

$$\text{size of synthesis areas} = (A)/(S)$$

where:

A is the total area available for synthesis; and

S is the number of sequences desired in the area.

S_ℓ will generally be less than or equal to n, unless duplicate sequence areas are desired.

It will be appreciated by those of skill in the art that the above method could readily be used to simultaneously produce thousands or millions of oligomers on a substrate using conventional photolithographic techniques. Consequently, the method results in the ability to practically test large numbers of, for example, penta, hexa, hepta, or even octapeptides.

The above example has illustrated the method by way of a manual example. It will of course be appreciated that automated or semi-automated methods could be used. The substrate would be mounted in a flow cell for automated addition and removal of reagents, to minimize the volume of reagents needed, and to more carefully control reaction conditions. Successive masks could be applied manually or automatically.

In synthesizing the dimer of an aminopropyl group and a fluorescent group, a functionalized durapore membrane was used as a substrate. The durapore membrane was a polyvinylidine difluoride with aminopropyl groups. The aminopropyl groups were protected with the DDZ group by reaction of the carbonyl chloride with the amino groups, a reaction readily known to those of skill in

the art. The surface bearing these groups was placed in a solution of THF and contacted with a mask bearing a checkerboard pattern of 1 mm opaque and transparent regions. The mask was exposed to ultraviolet light having a wavelength down to at least about 280 nm for about 5 minutes at ambient temperature, although a wide range of exposure times and temperatures may be appropriate in various embodiments of the invention. For example, in one embodiment, an exposure time of between about 1 and 5000 seconds may be used at process temperatures of between -70 and +50°C.

In one preferred embodiment, exposure times of between about 1 and 500 seconds at about ambient pressure are used. In some preferred embodiments, pressure above ambient may be used to prevent evaporation.

The surface of the membrane was then washed for about 1 hour with a fluorescent label which included an active ester bound to a chelate of a lanthanide. Wash times will vary over a wide range of values from about a few minutes to a few hours. These materials fluoresce in the red and the green visible region. After the reaction with the active ester in the fluorophore was complete, the locations in which the fluorophore was bound could be visualized by exposing them to ultraviolet light and observing the red and the green fluorescence. It was observed that the substrate closely followed the original pattern of the mask.

V. Screening

One application of the above-described method lies in screening for biological activity.

A substrate prepared as discussed above would be exposed to a marked receptor such as an antibody. The ligand could be marked in any one of a variety of ways, but in one embodiment the receptor is marked with a radioactive marker. The receptor is contacted with the above-described substrate, and placed on or in the prox-

imity of x-ray film. Alternatively, a fluorescent marker may be provided and detection may be by way of a charge-coupled device (CCD).

In the use of autoradiography, the marker is a radioactive label, such as ^{32}P. The marker is exposed to film, which is developed, then read out on a scanner. An exposure time of about 1 hour would be required for autoradiography in one embodiment. Fluorescence detection using, for example, a fluorophore such as fluorescence attached to the receptor will usually require shorter exposure, e.g., 1 second or less.

Use of the invention herein is illustrated primarily with reference to screening for biological activity. The invention will, however, find many other uses. For example, the invention may be used in information storage (e.g., on optical disks), production of molecular electronic devices, production of stationary phases in separation sciences, and in immobilization of cells, proteins, lectins, nucleic acids, polysaccharides and the like in patterns on a surface via molecular recognition of specific peptide sequences. By synthesizing the same compound in progressively layered areas a gradient is established to control chemotaxis or to develop diagnostic dipsticks which titrate an antibody against an increasing amount of antigen. By synthesizing several catalyst molecules in close proximity, more efficient multistep conversions may be achieved by "coordinate immobilization." Coordinate immobilization also may be used for electron transfer systems, as well as to provide both structural integrity and other desirable properties to materials such as lubrication, wetting, etc.

It is to be understood that the above description is intended to be illustrative and not restrictive. Many embodiments will be apparent to those of skill in the art upon reviewing the above description. By way of example, the invention has been described primarily with

reference to the use of photoremovable protective groups, but it will be readily recognized by those of skill in the art that sources of radiation other than light could also be used. For example, in some embodiments it may be desirable to use protective groups which are sensitive to electron beam irradiation, x-ray irradiation, in combination with electron beam lithograph, or x-ray lithography techniques. Alternatively, the group could be removed by exposure to an electric field. The scope of the invention should, therefore, be determined not with reference to the above description, but should instead be determined with reference to the appended claims, along with the full scope of equivalents to which such claims are entitled.

WHAT IS CLAIMED IS:

1. A method of synthesizing a plurality of chemical sequences, said chemical sequences comprising at least a first and a second subunit, comprising the steps of:

a) at a first area on a substrate having at least a first and a second area, said first and said second area comprising a protective group, removing said protective group in said first area;

b) exposing said first subunit to said substrate, said first subunit further comprising a first subunit protective group, said first subunit binding at said first area;

c) activating second area to remove said protective group in said second area;

d) exposing said second subunit to said substrate, said second subunit further comprising a second subunit protective group, said second subunit binding at said second area;

e) activating first area to remove said first subunit radiation-sensitive protective group;

f) providing a third subunit to said substrate, said third subunit binding at said first area to produce a first sequence;

g) activating said second area to remove said second subunit radiation-sensitive protective group; and

h) exposing a fourth subunit to said substrate, said fourth subunit binding at said second area to produce a second sequence, said second sequence different from said first sequence.

2. A method of synthesizing a plurality of chemical sequences, said chemical sequences comprising at least a first and a second subunit, comprising the steps of:

a) on a substrate having at least a first and a second area deactivating said first area to provide a first protective group in said first area;

b) exposing said first subunit to said substrate, said first subunit binding at said second area;

c) removing said protective group in said first area;

d) deactivating said second area to provide a second protective group in said second area;

e) exposing said second subunit to said substrate, said second subunit binding at said first area;

f) removing said protective group in said second area;

g) deactivating said first area to provide a protective group in said first area;

h) exposing a third subunit to said substrate, said third subunit binding at said second area to produce a first sequence;

i) removing said protective group in said first area; and

j) exposing a fourth subunit to said substrate, said subunit binding at said first area to produce a second sequence, said second sequence different than said first sequence.

3. A method of synthesizing at least a first polymer sequence and a second polymer sequence on a substrate, said first polymer having a different monomer sequence from said second polymer, comprising the steps of:

a) inserting a first mask between said substrate and an energy source, said mask having first regions and second regions, said first regions permitting passage of energy from said source, said second regions blocking energy from said source;

b) directing energy from said source at said substrate, said energy removing a protective group under said first regions;

c) exposing a second portion of said first polymer to said substrate to create a first sequence;

d) inserting a second mask between said substrate and said energy source, said second mask having first regions and second regions;

e) directing energy from said source at said substrate, said energy removing said protective group under said first regions of said second mask; and

f) exposing a second portion of said second polymer to said substrate, said second portion of said second polymer binding with said first portion of said second polymer to create a second sequence.

4. The method as recited in Claim 3, wherein said energy is selected from the group ion beams, electron beams, gamma rays, x-rays, ultra-violet radiation, light, infra-red radiation, microwaves, electric fields, radio-waves, and combinations thereof.

5. The method as recited in Claim 1, wherein the protective groups are photosensitive protective groups.

6. The method as recited in Claims 1 or 2, wherein said steps of activating are steps of applying light to selected regions of said substrate.

7. The method as recited in Claims 1 or 2, wherein the first and the second subunits are amino acids.

8. The method as recited in Claims 1, 2 or 3 further comprising the step of screening said first and said second sequence for affinity with a receptor, said step of screening further comprising the step of exposing said substrate to said receptor and testing for the presence of said receptor.

9. The method as recited in Claim 8, wherein the step of screening is a step of screening with a member selected from the group antibodies, receptors having specific binding affinity.

10. The method as recited in Claims 1, 2 or 3, wherein the substrate is selected from the group consisting of a polymerized Langmuir Blodgett film, functionalized glass, germanium, silicon, polymers, (poly)tetrafluoroethylene, gallium arsenide, gallium phosphide, silicon oxide, silicon nitride and combinations thereof.

11. The method as recited in Claim 1, wherein the protective group, the first subunit protective group, and the second subunit protective group are selected from the group consisting of ortho-nitrobenzyl derivatives, 6-nitroveratryloxycarbonyl, 2-nitrobenzyloxycarbonyl, and mixtures thereof.

12. The method as recited in Claims 1 or 2, wherein the first and second areas each have total areas of less than 1 cm^2.

13. The method as recited in Claims 1 or 2, wherein the first and the second areas each have total areas of between about 1 μm^2 and 100 μm^2.

14. The method as recited in Claim 6, wherein the light is monochromatic coherent light.

15. The method as recited in Claims 1 or 2, wherein the steps of activating are carried out with a solution in contact with said substrate.

16. The method as recited in Claim 15, wherein said solution further comprises said third or said fourth subunit.

17. The method as recited in Claim 8, wherein the receptor further comprises a marker selected from the group radioactive markers and fluorescent markers and wherein said step of testing for the presence of the receptor is a step of detecting said marker.

18. The method as recited in Claims 1 or 2, wherein at least two of said first, said second, said third, and said fourth subunits are the same.

19. A method of producing a plurality of polymers comprising the steps of:

a) on a material comprising at least a first portion of a first polymer at a first location and a first portion of a second polymer at a second location, said first portions having a removable protective group irradiating said first location to remove said protective group from said first portion of said first polymer;

b) exposing said material to a second portion of said first polymer, said second portion of said first polymer reacting with said first portion of said first polymer, said second portion further comprising a removable protective group;

c) irradiating said second location to remove said protective group from said first portion of said second polymer; and

d) exposing a second portion of said second polymer, said second portion of said second polymer reacting with said first portion of said second polymer, said second portion further comprising a removable protective group.

20. A method of identifying at least one peptide sequence for binding with a receptor comprising the steps of:

a) on a substrate having a plurality of polypeptides having a photoremovable protective group irradiating first selected polypeptides to remove said protective group;

b) contacting said polypeptides with a first amino acid to create a first sequence, said first amino acid further comprising a protective group;

c) irradiating second selected polypeptides to remove said protective group;

d) contacting said polypeptides with a second amino acid to create a second sequence;

e) contacting said first and second sequence with said receptor; and

f) identifying which of said first or said second sequence binds with said recognition protein.

21. The method as recited in Claim 20, wherein the step of identifying further comprises the step of identifying the presence of a marker selected from the

group radioactive markers and fluorescent markers in said receptor.

22. Apparatus for screening for biological activity comprising:

a) a substrate comprising a plurality of polymer sequences, said polymer sequences attached to a surface of said substrate at a known location on said substrate, each of said sequences occupying an area of less than about 10000 μm^2;

b) means for exposing said substrate to a receptor, said receptor marked with a radioactive marker, said receptor binding with at least one of said sequences; and

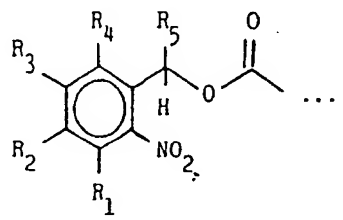
c) means for detecting a location of said radioactive marker on said substrate.

23. Apparatus for preparation of a plurality of sequences comprising:

a) a substrate with a surface, said surface comprising a reactive portion, said reactive portion activated upon exposure to an energy source so as to react with a monomer of the sequence; and

b) means for selectively protecting and exposing portions of said surface from said energy source.

24. Apparatus as recited in Claim 23, wherein said reactive portion further comprises a protective group, said protective group of the form:



often limited by the number and type of ligands which are available. Novel ligands are sometimes discovered by chance or by application of new techniques for the elucidation of molecular structure, including x-ray crystallographic analysis and recombinant genetic techniques for proteins.

Small peptides are an exemplary system for exploring the relationship between structure and function in biology. A peptide is a short sequence of amino acids. When the twenty naturally occurring amino acids are condensed into polymeric molecules they form a wide variety of three-dimensional configurations, each resulting from a particular amino acid sequence. The number of possible pentapeptides is 20^5 or 3.2 million different peptides. The likelihood that molecules of this size might be useful in receptor-binding studies is supported by epitope analysis studies showing that some antibodies recognize sequences as short as 5-8 amino acids with high specificity. Furthermore, the average molecular weight of 100 per amino acid puts small peptides in the size range of many currently useful pharmaceutical products.

Pharmaceutical drug discovery is one type of research which relies on such a study of SAR. In most cases, contemporary pharmaceutical research can be described as the process of discovering novel ligands with desirable patterns of specificity for biologically important receptors. Another example is research to discover new compounds for use in agriculture, such as pesticides and herbicides.

Sometimes, the solution to a rational process of designing ligands is difficult or unyielding. In every case, the process of obtaining the necessary structural detail on the receptor is tedious and expensive, primarily because prior methods of preparing large numbers of different polymers have been painstakingly slow when used at a scale sufficient to permit effective

where R_1 is alkoxy, alkyl, halo, aryl, alkenyl, or hydrogen; R_2 is alkoxy, alkyl, halo, aryl, nitro, or hydrogen; R_3 is alkoxy, alkyl, halo, nitro, aryl, or hydrogen; R_4 is alkoxy, alkyl, hydrogen, aryl, halo, or nitro; and R_5 is alkyl, alkinyl, cyano, alkoxy, hydrogen, halo, aryl, or alkenyl.

25. Apparatus as recited in Claim 23, wherein said reactive portion further comprises linker molecules, said linker molecules selected from the group ethylene glycol oligomers, diamines, diacids, amino acids, or combinations thereof.

26. Apparatus as recited in Claim 23, wherein said means for selectively protecting further comprises a mask.

27. Apparatus as recited in Claim 23, wherein said energy source is a light source.

28. Apparatus as recited in Claim 23, wherein said reactive portion further comprises a composition selected from the group nitroveratryloxy carbonyl, nitrobenzyloxy carbonyl, dimethyl-dimethoxybenzyloxy carbonyl, 5-bromo-7-nitroindolinyl, hydroxy-2-methyl cinnamoyl, and 2-oxymethylene anthraquinone.

29. A method of preparing a substrate comprising the steps of:

a) exposing a first area of said substrate to an activator to remove a protective group;

b) exposing at least said first area to a first monomer;

c) exposing a second area to an activator to remove a protective group; and

d) exposing at least said second area to a second monomer.

30. The method as recited in Claim 29, wherein said steps of exposing to an activator uses an activator selected from the group ion beams, electron beams, gamma rays, x-rays, ultra-violet radiation, light, infra-red radiation, microwaves, electric currents, radiowaves, and combinations thereof.

31. The method as recited in Claim 29, wherein the protective groups are photosensitive protective groups.

32. The method as recited in Claim 29, wherein said steps of exposing to an activator are steps of applying light to selected regions of said substrate.

33. The method as recited in Claim 29, wherein the first and the second subunits are amino acids.

34. The method as recited in Claim 29 further comprising the step of screening sequences on said substrate for affinity with a receptor, said step of screening further comprising the step of exposing said substrate to said receptor and testing for the presence of said receptor in said first and said second area.

35. The method as recited in Claim 34, wherein the step of screening is a step of screening with antibodies.

36. The method as recited in Claim 29, wherein the substrate is selected from the group polymerized Langmuir Blodgett film, functionalized glass, germanium, silicon, polymers, (poly)tetrafluoroethylene, gallium arsenide, and combinations thereof.

37. The method as recited in Claim 29, wherein the protective group is selected from the group ortho-nitrobenzyl derivatives, 6-nitroveratryloxycarbonyl, 2-nitrobenzyloxycarbonyl, and mixtures thereof.

38. The method as recited in Claim 29, wherein the first and second areas each have total areas of less than 1 cm^2.

39. The method as recited in Claim 29, wherein the first and the second areas each have total areas of between about 1 μm^2 and 100 μm^2.

40. The method as recited in Claim 32, wherein the light is monochromatic coherent light.

41. The method as recited in Claim 29, wherein the steps of exposing to an activator are carried out with a solution in contact with said substrate.

42. The method as recited in Claim 41, wherein said solution further comprises said first or said second subunit.

43. The method as recited in Claim 34, wherein the ligand further comprises a marker selected from the group radioactive markers and fluorescent markers and wherein said step of testing for the presence of the receptor is a step of detecting said marker.

44. The method as recited in Claim 29, wherein the steps of exposing to an activator further comprise the steps of:

a) placing a mask adjacent said substrate, said mask having substantially transparent regions and substantially opaque regions at a wavelength of light; and

b) illuminating said mask with a light source, said light source producing at least said wavelength of light.

45. The method as recited in Claim 29, wherein said steps are repeated so as to synthesize 10^3 or more different sequences on said substrate.

46. The method as recited in Claim 29, wherein said steps are repeated so as to synthesize 10^4 or more different sequences on said substrate.

47. Apparatus for preparation of a substrate having a plurality of amino acid sequences thereon, said apparatus comprising:

a) a substrate with a surface;

b) a protective group on said surface said protective group removable upon exposure to an energy source, said energy source selected from the group consisting of light, electron beams, and x-ray radiation;

c) means for directing said energy source at selected locations on said surface; and

d) means for exposing monomers to said surface for binding to said surface.

48. Apparatus for screening polymers comprising a substrate with a surface, said surface comprising a plurality of amino acid sequences at known locations, each of said sequences occupying an area of less than about 1 cm^2.

49. Apparatus as recited in Claim 48, wherein said area is less than 10000 μm^2.

50. Apparatus as recited in Claim 30, wherein said area is less than about 100 μm^2.

51. A method for screening a plurality of polymers for biological activity comprising exposing a receptor to a substrate having said plurality of said polymers on a surface thereof, each of said polymers occupying an area of less than about 1 cm^2.

52. A method for screening as recited in Claim 51, wherein said area is less than about 10000 μm^2.

53. A method as recited in Claim 51, wherein said area is less than about 100 μm^2.

54. A substrate for screening for biological activity, said substrate comprising 10^3 or more different ligands, said ligands having known locations.

55. A substrate as recited in Claim 54, wherein said substrate comprises 10^6 or more different ligands.

56. A substrate as recited in Claim 54, wherein the ligands are peptides.

57. A method of synthesizing up to n^ℓ different types of polymers, each polymer being made up of ℓ monomers selected from a set of n different types of monomers, where n and ℓ are greater than 1, said method comprising:

a) attaching a plurality of one type of monomer from said set to at least a portion of a substrate to form a first layer of said first type of monomer, said first layer having a surface made up of n portions, said monomers having a protective material which prevents additional monomers from attaching to said first layer of monomers;

b) removing said protective material from a first portion of the surface of said first layer;

c) attaching a first selected type of monomer form said set of n monomers to the monomers exposed by removal of the protective material to form a second layer of said first selected type of monomer over those portions of the monomers of said first type exposed by removal of said protective material; and

d) repeating steps b) and c) up to (n-1) times.

58. The method of Claim 57, wherein each of said monomers in said second layer is provided with a protective material which prevents additional monomers from attaching to the monomers in said second layer.

VERY LARGE SCALE IMMOBILIZED POLYMER SYNTHESIS

ABSTRACT OF THE DISCLOSURE

A method and apparatus for preparation of a substrate containing a plurality of polymer sequences is disclosed. Photoreactive protective groups are attached to a surface of a substrate. Selected portions of the substrate are exposed to light so as to activate the selected areas. A chemical subunit, also containing a photoreactive protective group is provided to the substrate to bind at the selected areas. The process is repeated using a variety of chemical subunits such as amino acids until sequences of a desired length are obtained. Detection methods using fluorescent and radioactive detectors are also disclosed.

ATTORNEY DOCKET NO.

DECLARATION AND POWER OF ATTORNEY

As a named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor, (if only one name is listed below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

VERY LARGE SCALE IMMOBILIZED POLYMER SYNTHESIS

the specification of which ☒ is attached hereto or ☐ was filed on _____ as Application Serial No. _____ and was amended on _____ (if applicable).

I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a). I claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)

COUNTRY	APPLICATION NUMBER	DATE OF FILING	PRIORITY CLAIMED UNDER 35 U.S.C. 119
			Yes____ No____
			Yes____ No____

I claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	DATE OF FILING	STATUS
		☐ Patented ☐ Pending ☐ Abandoned
		☐ Patented ☐ Pending ☐ Abandoned

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) who are partners and associates in the firm of Townsend and Townsend to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

Vern Norviel, Reg. No. 32,483
William M. Smith, Reg. No. 30,223

SEND CORRESPONDENCE TO:	DIRECT TELEPHONE CALLS TO: (name, registration number, and telephone number)
Vern Norviel TOWNSEND and TOWNSEND Steuart Street Tower, One Market Plaza San Francisco, CA 94105	Vern Norviel Reg. No. 32,483 ☐ (415) 543-9600 or ☒ (415) 326-2400

201	FULL NAME OF INVENTOR	Last Name: PIRRUNG	First Name: MICHAEL	Middle Name or Initial: C.	
	RESIDENCE & CITIZENSHIP	City: Menlo Park	State or Foreign Country: California	Country of Citizenship: U.S.A.	
	POST OFFICE ADDRESS	Post Office Address: 2161 Avy Avenue,	City: Menlo Park,	State or Country: California	Zip Code: 94025
202	FULL NAME OF INVENTOR	Last Name: READ	First Name: J.	Middle Name or Initial: LEIGHTON	
	RESIDENCE & CITIZENSHIP	City: Palo Alto	State or Foreign Country: California	Country of Citizenship: U.S.A.	
	POST OFFICE ADDRESS	Post Office Address: 1001 Ramona,	City: Palo Alto,	State or Country: California	Zip Code: 94301
203	FULL NAME OF INVENTOR	Last Name:	First Name:	Middle Name or Initial:	
	RESIDENCE & CITIZENSHIP	City:	State or Foreign Country:	Country of Citizenship:	
	POST OFFICE ADDRESS	Post Office Address:	City:	State or Country:	Zip Code:

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature of Inventor 201	Signature of Inventor 202	Signature of Inventor 203
Michael C. Pirrung	J. Leighton Read	
Date: 6/7/89	Date: 6/1/89	Date:

Atty. D- .o. 11509-9

#4

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(f) and 1.27(c)) – SMALL BUSINESS CONCERN

Applicant or Patentee: Michael C. Pirrung et al.
Serial No.: 362,901 Filing Date: June 7, 1989
Patent No.: Issued:
For: VERY LARGE SCALE IMMOBILIZED POLYMER SYNTHESIS

I hereby declare that I am

[ ] the owner of the small business concern identified below:
[X] an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN AFFYMAX N.V., a Netherlands corporation
ADDRESS OF CONCERN Van Boshuizenstraat 12
1083 BA Amsterdam, The Netherlands

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled VERY LARGE SCALE IMMOBILIZED POLYMER SYNTHESIS by inventor(s) Michael C. Pirrung and J. Leighton Read described in

[ ] the application filed herewith
[X] application serial no. 362,901, filed June 7, 1989.
[ ] patent no. , issued .

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

NAME
ADDRESS
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

NAME
ADDRESS
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING J. Leighton Read
TITLE OF PERSON OTHER THAN OWNER Managing Director
ADDRESS OF PERSON SIGNING 1001 Ramona, Palo Alto, CA 94301

SIGNATURE L Read DATE 7/17/89

As Original Filed

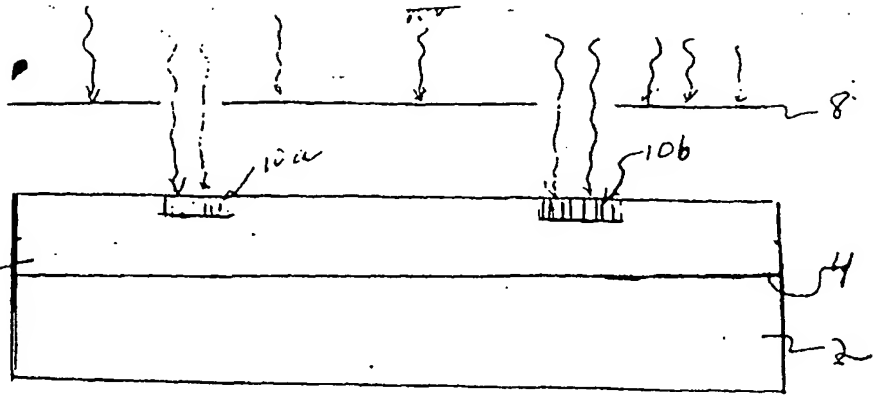


FIG. 1

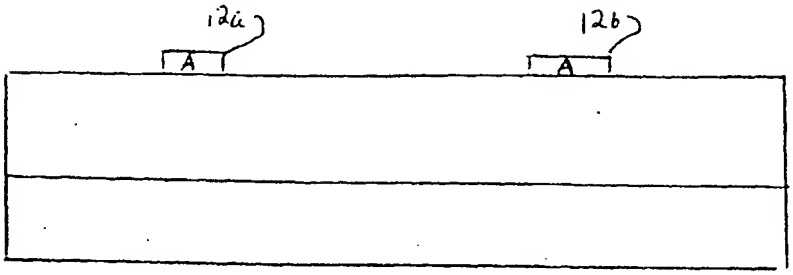


FIG. 2

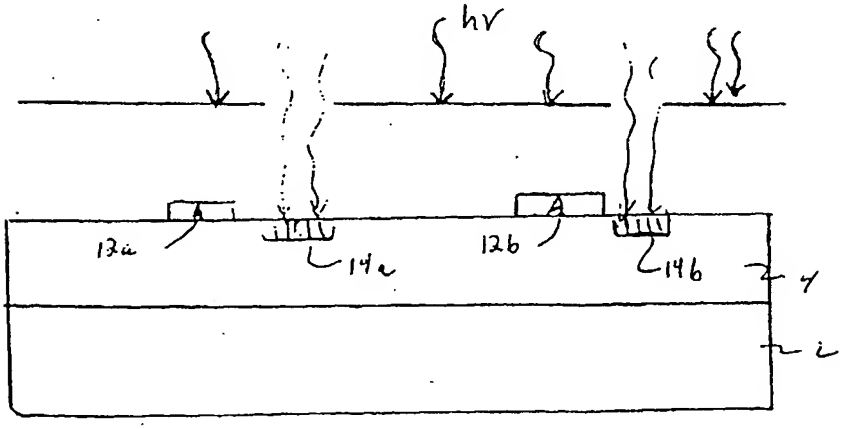


FIG. 3

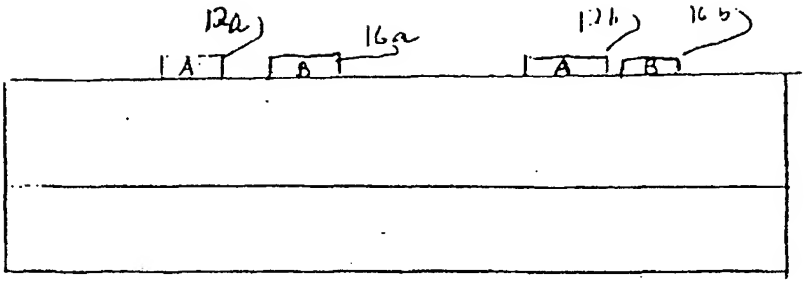


FIG. 4

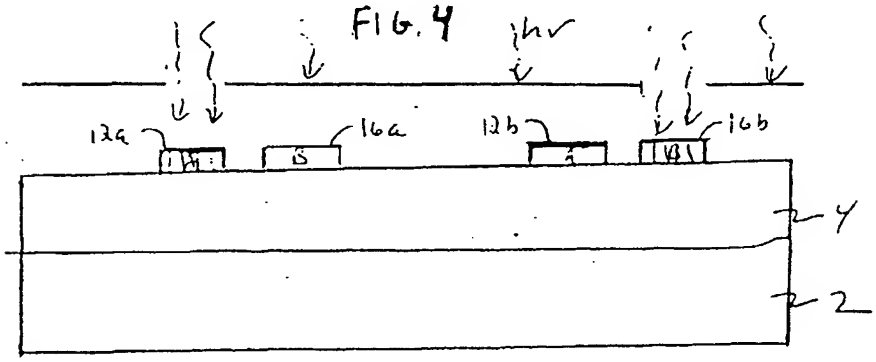


FIG. 5

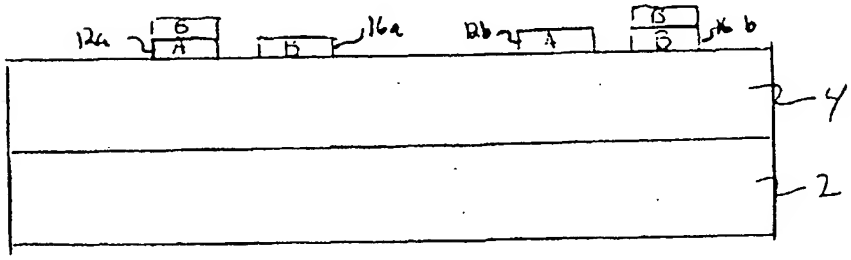


FIG. 6

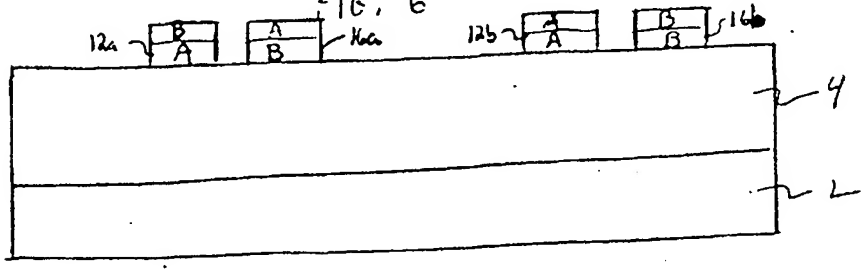


FIG. 7

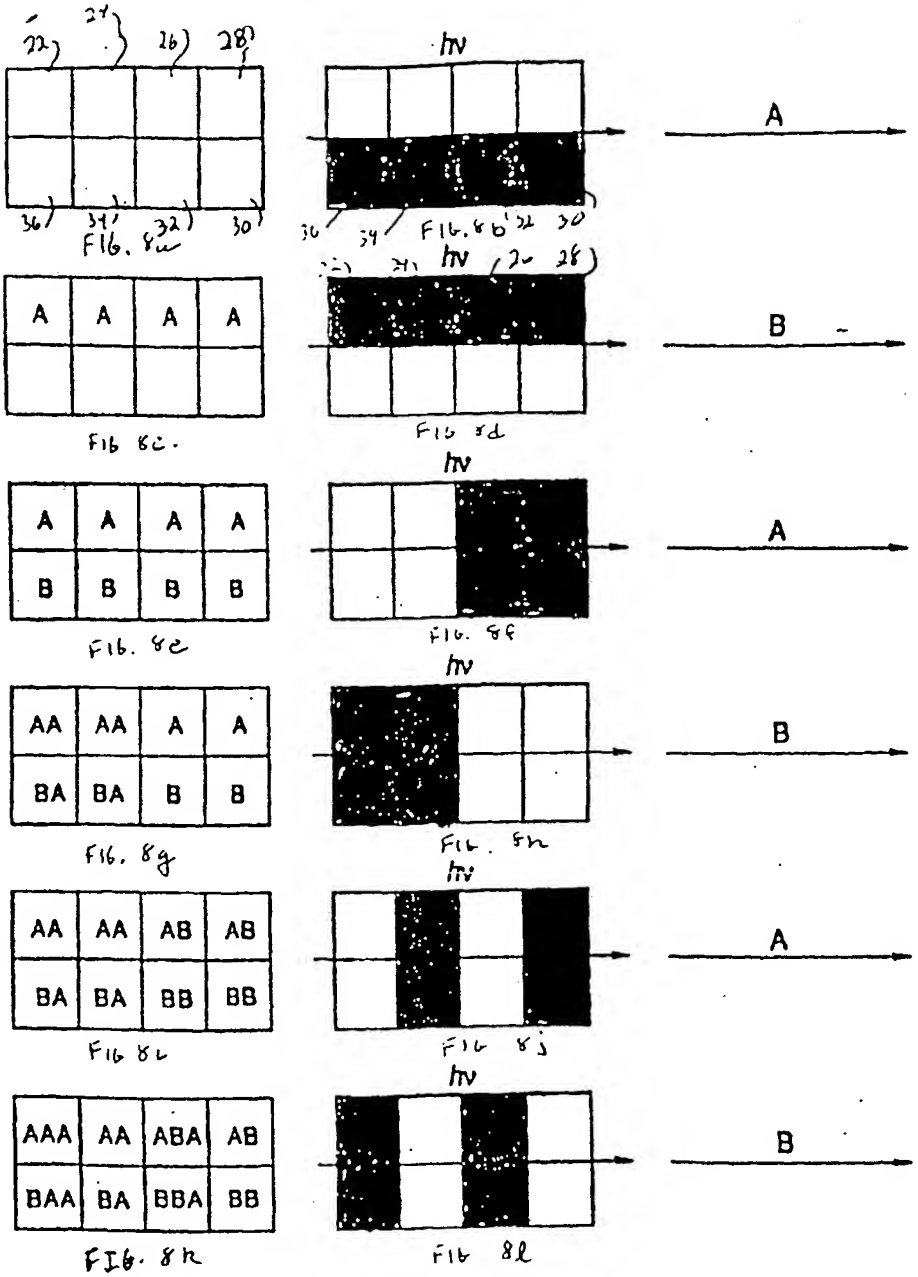


Fig. 8a

Fig. 8b

Fig. 8c

Fig. 8d

Fig. 8e

Fig. 8f

Fig. 8g

Fig. 8h

Fig. 8i

Fig. 8j

Fig. 8k

Fig. 8l

Fig. 8m